

(a) (i) transfected the cell with a first vector that expresses a replication factor; or

(ii) otherwise obtaining a cell that expresses or will express the replication factor; and

(b) transfected the cell with a second vector, wherein

(i) the second vector contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the [DNA] selectable marker;

(ii) the second vector additionally contains a second DNA in operative combination with a promoter for expression of the second DNA, and which second DNA does not code for a selectable marker; and

(iii) extrachromosomal replication of the second vector is dependent upon presence within the cell of the replication factor.

3. A method according to Claim 2 wherein the viral replication factor is selected from polyoma large T antigen, EBNA-1 antigen, papilloma virus replication factors, SV40 large T antigen and functional variants, analogues and derivatives thereof appropriate to the cell species.

4. A method according to Claim 1 wherein the second vector does not express the replication factor.

5. A method according to Claim 1 wherein the selectable marker is an antibiotic resistance gene.

6. A method according to Claim 1 further comprising transfected the cell with a third vector, wherein the third vector contains a DNA, or is adapted to receive a DNA.

in operative combination with a promoter for expression of the DNA, and replication of the third vector is dependent upon presence within the cell of the replication factor.

8. A method according to Claim 1 wherein the cell is selected from the group consisting of a mammalian cell and an avian cell.

9. A method according to Claim 1 wherein the cell is an embryonic cell.

11. A method according to Claim 1 for transfection of an ES cell wherein the ES cell of step (a) expresses polyoma large T antigen and the second vector comprises a natural target for polyoma large T antigen.

12. A method according to Claim 1 wherein the DNA codes for a polypeptide or protein.

13. A method according to Claim 1 wherein the DNA codes for an antisense RNA.

14. A method according to Claim 1 wherein the promoter is inducible.

15. A method according to Claim 1 wherein transcription of the DNA can be activated by a site specific recombinase.

16. A method according to Claim 1 wherein replication of the second vector can be prevented by a site specific recombinase.

17. A vector for transfection of a pluripotent cell *in vitro*, wherein:

- (i) the vector contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker;
- (ii) the vector contains a second DNA in operative combination with a promoter for expression of the DNA, and which second DNA does not code for a selectable marker;

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(iii) extrachromosomal replication of the vector is dependent upon presence within the cell of a replication factor; and

(iv) the vector does not express the replication factor.

19. A vector according to ~~Claim 18~~ wherein the viral replication factor is selected from the group consisting of polyoma large T antigen, EBNA-1 antigen, papilloma virus replication factors, SV40 large T antigen and functional variants, analogues and derivatives thereof.

20. A vector according to ~~Claim 17~~ wherein the vector is [substantially] free of DNA coding for the replication factor or any part thereof.

21. A vector according to ~~Claim 17~~ for transfection of mammalian or avian cells.

22. A vector according to ~~Claim 17~~ for transfection of ES cells.

23. A vector according to ~~Claim 22~~ comprising a natural target for polyoma large T antigen.

24. A vector according to ~~Claim 17~~ wherein the DNA codes for a polypeptide or protein.

25. A vector according to ~~Claim 17~~ wherein the DNA codes for an antisense DNA.

26. A vector according to ~~Claim 17~~ wherein the promoter is inducible.

27. A vector according to any ~~Claim 17~~ wherein the selectable marker is an antibiotic resistance gene.

29. An ES, EC or EG cell transfected with a first vector that expresses a replication factor and with a second vector according to ~~Claim 17~~.

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32. A cell selected from an ES, EC or EG cell according to Claim 29, and differentiated progeny thereof.

33. An assay for the effect of presence in a pluripotent cell of a protein or polypeptide or other product of DNA expression, comprising the steps:

(a) (i) transfecting the cell with a first vector that expresses a replication factor; or

(ii) otherwise obtaining a cell that expresses or will express the replication factor;

(b) transfecting the cell with a second vector, wherein

(i) the second vector contains a DNA coding for the protein or polypeptide or other product of DNA expression in operative combination with a promoter for expression of the DNA;

(ii) the second vector also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker; and

(iii) extrachromosomal replication of the second vector is dependent upon presence within the cell of the replication factor;

(c) selecting for cells that have been transfected with the second vector; and

(d) maintaining the selected cells over a plurality of generations so as to assay the effect of expression of the protein or polypeptide or other product of DNA expression.

35. An assay according to Claim 33 for assay of the effect of presence in the cell of two factors, each factor being independently selected from a protein, a polypeptide and another product of DNA expression.

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36. A method of screening a library of cDNAs comprising assaying the effect of expression of each of the cDNAs according to the method of Claim 33.

37. A method of investigating the properties of a DNA sequence comprising expressing in a pluripotent cell a composite DNA including (a) the DNA sequence under investigation, linked to (b) a DNA coding for a cell active protein, wherein

(i) activity of the cell active protein is dependent upon transport of the cell active protein to the cell surface,

(ii) the DNA of (b) does not code for a polypeptide that direct[ing]s transportation of the cell active protein to the cell surface, and

(iii) the cell active protein inhibits differentiation of the cell and in the absence of the cell active protein the cell will differentiate.

39. A method according to Claim 37 wherein the DNA of (b) is obtained by deleting or disabling, from a DNA encoding a cell surface or secreted protein, that portion of the DNA that codes for the polypeptide sequence responsible for transportation of the protein to the cell surface.

40. A method according to Claim 37 wherein the cell active protein induces a morphological or proliferative change in the cell.

42. A method according to Claim 37 wherein the cell active protein is a cell surface receptor.

44. A method according to Claim 37 comprising investigating the properties of a DNA in mammalian or avian cells.

45. A method according to Claim 37 comprising investigating the properties of a DNA in embryonic cells.

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47. A method according to Claim 37 comprising expressing the composite DNA by :

- (a) (i) transfecting the cell with a first vector that expresses a replication factor; or
- (ii) otherwise obtaining a cell that expresses or will express the replication factor;
- (b) transfecting the cell with a second vector, wherein
 - (i) the second vector contains the composite DNA in operative combination with a promoter for expression of the composite DNA;
 - (ii) the second vector also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker; and
 - (iii) extrachromosomal replication of the second vector is dependant upon presence within the cell of the replication factor;
- (c) selecting for cells that have been transfected with the second vector; and
- (d) maintaining the selected cells over a plurality of generations so as to assay the effect of expression of the composite DNA.

48. A method according to Claim 37 wherein step (a) is carried out once and the cells obtained are divided and used for a plurality of separate methods in which steps (b)-(d) are carried out a plurality of times with second vectors containing different DNA sequences.

49. A method according to Claim 37 for identification of a DNA coding for a cell surface or secreted protein.

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